

Cloning and expression of *MP13* gene from rat hippocampus, a new factor related to guanosine triphosphate regulation

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Abstract

C-Fos and the *Fos*-related antigens (*FRA*) are induced by various stimuli. A novel 35–37 kDa *FRA* was induced much longer after the treatment using kainic acid (KA) and may be very important for neuronal survival after brain damage. To identify this long-term *FRA*, we have constructed a cDNA library derived from hippocampus after KA treatment and screened it with an antibody highly conserved M-peptide region of *FRAs*. One gene, *MP13*, was cloned with a 1662 bp open reading frame and coded for a 554-amino acid protein. *MP13* has a leucine zipper region, a glutamine repeat region, and has high similarity to the activator of the small guanosine triphosphate (GTP)ase *Rab5*. Gel retardation analysis revealed that *MP13* functions as a GTP regulation related factor. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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The induction of *c-Fos* and the *Fos*-related antigens (*FRA*), including *Fos B*, *Fra-1* and *Fra-2*, by various stimuli has been extensively studied [1,3,4,6,9]. In most cases, the induction of *FRA* are rapid and transient, lasting about 1–4 h after the stimuli [7,8]. However, several laboratories including ours have described a novel 35–37 kDa *FRA* which is induced for a much longer time after stimulation and whose distribution is distinct from the induction of other *FRAs*. This long-term elevated *FRA* can be induced by the chronic administration of cocaine [6,10] or morphine [6]. We have found that systemic administration of kainic acid (KA), a glutamate analog, induced a 35 kDa *FRA* that persisted for up to 5 months in the granule cells of the dentate gyrus [1,5,9]. The administration of KA not only caused epileptic seizures but also damaged certain areas of the rat brain, especially the hippocampus where axonal sprouting from the granule cells of the dentate gyrus occurred. The long-lasting changes of *FRA* expression, thus, may underlie these long-term effects of the KA. This long-term expression of

FRA after KA treatment suggests that the granule cells of the dentate gyrus remain activated for a protracted time, which presumably reflects permanent changes in their genomic programming. The gene encoding for this *FRA* protein, therefore, may be very important both in the brain's adaptation to cocaine and morphine, and in neuronal survival and sprouting after brain damage.

In order to identify this long-term *FRA*, we have constructed a cDNA library derived from the hippocampus 3 days after KA treatment. One of 41 positive clones, *MP13*, from 2 million screened colonies was selected. We now report the cloning and expression of *MP13*, which may functionally relate to guanosine triphosphate (GTP) regulation.

The KA-treated rat hippocampal cDNA library was constructed with the Lambda ZAP cDNA synthesis kits (Stratagene, La Jolla, CA). Adult male Fischer rats (250–300 g, Charles River, Raleigh, NC) were injected intraperitoneally (i.p.) with KA (8 mg/kg; 1 ml/kg) or saline (control). Only the animals with convulsive behavior (forelimb clonus with intermittent episodes of whole-body clonus) within 4 h following KA administration were used for this study. Rats were sacrificed by decapitation 3 days

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The *Fos*-related antigen (*FRA*) gene was screened with the antibody generated against a highly conserved sequence of the M-peptide of the *FRA* [3,6] using the PicoBlue™ Immunoscreening Kit (Stratagene, La Jolla, CA). Colonies were seeded onto LB plates supplemented with 2 mM IPTG and transferred onto a nitrocellulose membrane. The membranes were blocked in a Tris–NaCl/Tween-20 buffer containing 3% skim milk and incubated at 4°C overnight with a 1:1000 dilution of *FRA* antiserum. After washing in

	300	310	320	330	340
Mp13	DELQEGLSRS	NEDCAKQMV	LLAQVQNSEQ	LLRTLQGTVS	QAQERVQORM
Rabaptin5	eELQqGLSqa	krDvqeQMav	LmsreQvSEe	LvR-LQkdnd	slQgkshlv
	620	630	640	650	660
	350	360	370	380	390
Mp13	AELATSHKCL	SOEVKRLNEE	NRGLRAEQLP	SSALQGSQQ	EDQDEALPSS
Rabaptin5	slqgaedfil	pdtteaLrEL	vlkyR-Edi-	invrtaadhv	EeklkAeilf
	670	680	690	700	710
	400	410	420	430	440
Mp13	IQELHQLVRH	TRQQAARQQ	AQEHEAERLR	IEIVKLREAL	DEETAAKASL
Rabaptin5	lkEqiQaegc	lkenleetlQ	leienckeel	asIssLkaeL	erikveKqgL
	720	730	740	750	760
	450	460	470	480	490
Mp13	EGQLRVQREE	TDVLEASLCS	LRIETERVQQ	EHKKAQLTDL	LSEQRAKALR
Rabaptin5	EstLReksqg	lesLq-ei-k	islEqklke	taaKatvegL	mfEeknKAqR
	770	780	790	800	810
	500	510	520	530	540
Mp13	LQAELETSEQ	VQRDFVRLSQ	ALQVRLEQIR	QADTLEQVRS	ILDEAPLRDI
Rabaptin5	LQtELdvSEQ	VQRDFVRLSQ	tLQVqLEIR	QADsLEriRA	ILndtKLTdI
	820	830	840	850	860
	550				
Mp13	KDIKDS*				
Rabaptin5	nqlpet				

Fig. 2. *MP13* peptide sequence was homologous to the C-terminal peptide sequence of rabaptin-5. Peptide homology searching was done with the Protein Identification Resource (PIR) database. M13 protein represents *MP13* peptide sequence; rabapti represents the rabaptin-5 peptide sequence; 'Λ' indicates the identity between the two amino acid residues; 'v' indicates that there are two different amino acid residues. The numbers indicate the positions of residues in the two sequences.

the Tris–NaCl buffer, the positive colonies were visualized using the alkaline phosphatase method. Forty one positive clones were isolated from 2×10^6 plaques. Polymerase chain reaction (PCR) was performed using primers conserved in all *FRA*. The predicted PCR fragments were found in one clone, *MP13*.

To further identify the clones, the fusion protein of *MP13* was tested by Western blot analysis. *MP13* was only recognized by *FRA* antibody, but not by *c-Fos*, *Fra-1*, or *Fra-2* antibodies (unpublished data). *MP13* and five other selected clones were used for further sequence analysis.

DNA samples for sequencing were prepared with Wizard™ Mini-Preps kits (Promega, Madison, WI) according to the manufacturer's protocol and 1 µg DNA was sequenced by the dideoxynucleotide chain termination method with Sequenase 2.0. DNA homology searching was done in the GenBank database with GCG software and the predicted amino acid sequences were compared with the Protein Identification Resource (PIR) database. *MP13* DNA sequence has been submitted to GenBank nucleotide sequence database with accession number U34932.

A complete cDNA sequence, 2221 bp, was generated from *MP13*. As shown in Fig. 1, the whole sequence contains an open reading frame of 1662 bp, a 60 bp 5'-non-coding region and a 500 bp 3'-non-coding sequence with a poly(A)⁺ tail. It codes for a protein of 554 amino

acid residues. This protein contains a series of four leucine heptad repeats which are separated by 111 amino acid residues from a series of four glutamine octet repeats. *MP13* protein has same peptide sequence except that the 5th amino acid was replaced by a Q (glutamine). The leucine repeats found in *MP13* protein resemble the leucine zipper found in all of known *FRAs*. However, *MP13* protein apparently lacks the basic DNA-binding region in all of known *FRAs*. This result suggests that *MP13* protein has a different function from the common function of the *FRA* protein. The function of four octet glutamine repeats is currently unknown. It warrants further investigation.

The homology searching revealed that two regions of the *MP13* protein sequence are similar to the C-terminus of *rabaptin-5* (Fig. 2), an activator of the small GTPase *Rab5* [12]. The first region extends from peptide position 298–335 and has more than 80% similarity to *rabaptin-5*. This region represents an α-turn point. The second region from peptide position 481 to the end has more than 90% similarity to *rabaptin-5*. Since *rabaptin-5* is a activator of small GTPase, the homologous searching result suggested that the function of *MP13* be related to the GTP regulation.

To identify the function of *MP13*, we translated *MP13* in vitro and tested the translated *MP13* protein in gel mobility-shift assay. The translation products of *MP13* DNA (0.1 µg; Fig. 3, lanes *MP13*) and vector SK (Fig. 3, lane SK) in vitro was generated by the Single Tube Protein™ system (Novagen, Madison, WI) using T3 RNA polymerase. The translation mixtures were analyzed by gel electrophoresis with (Fig. 3, Lanes +*FRA* Ab)/without immunoprecipitation with the *FRA* antibody, and with (Fig. 3, Lane +M

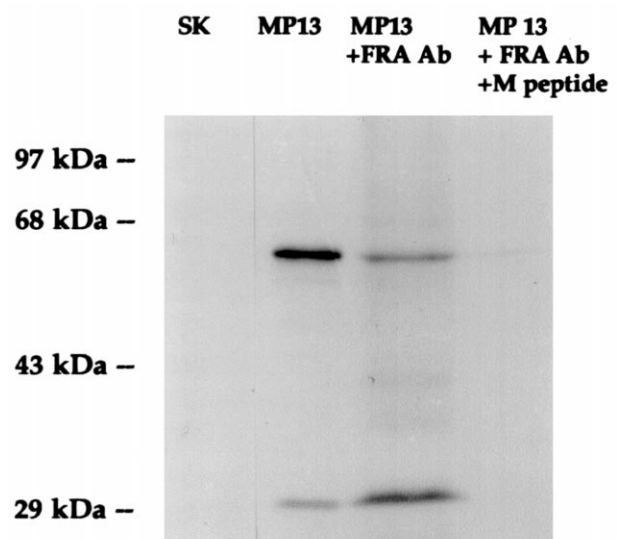


Fig. 3. Translation of *MP13* DNA in vitro. Translation of *MP13* DNA (0.1 µg; lanes *MP13*) and vector SK (lane SK) in vitro with the Single Tube Protein™ system using T3 RNA polymerase. The translation mixtures were analyzed by gel electrophoresis. The translation products of *MP13* cDNA was immunoprecipitation (*MP13*, + *FRA* Ab) with the *FRA* antibody, and with (lane +M peptide)/without M-peptide competition.

peptide)/without M-peptide competition. The results showed that *MP13* generated two major bands with apparent molecular weights of 62 and 32 kDa (Fig. 3). Both bands can be recognized by the *FRA* antibody and abolished by the competition with M-peptide, which showed that translated *MP13* products were especially recognized by *FRA* antibody. Next, we performed a non-denaturing gel mobility-shift assay to test whether the function of the *MP13* protein was related to GTP regulation. Translated *MP13* was bound to protein at room temperature for 20 min with/without the addition of GTP or GDT in binding mixture (20 mM Tris-HCl, pH 7.8, 100 mM NaCl, 5 mM MgCl₂, 1 mM EDTA, 5 mM DTT, 50 µg/ml bovine serum albumin, 100 µg/ml sonicated salmon sperm DNA, and 10% glycerol). Protein complexes were separated on a 5% non-denaturing polyacrylamide gel. Gels were run at 150 V in 50 mM Tris/50 mM boric acid/1mM EDTA, dried and autoradiographed. The results revealed that the addition of GDP did not affect the binding of *MP13* protein to other proteins (Fig. 4, lanes GDP +). However, the addition of GTP aborted the binding of *MP13* protein to other proteins (Fig. 4, lanes GTP +).

Sequence comparison revealed that part of the *MP13* protein is more than 90% similar to the C-terminus of the activator of the small GTPase *Rab5*, *rabaptin-5* [12]. The C-terminus of *rabaptin-5* is predicted to be mainly α -helical and contain heptad repeats characteristic of coiled-coil domains. It might interact with soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) that display coiled-coil domains [11] or it could be involved in

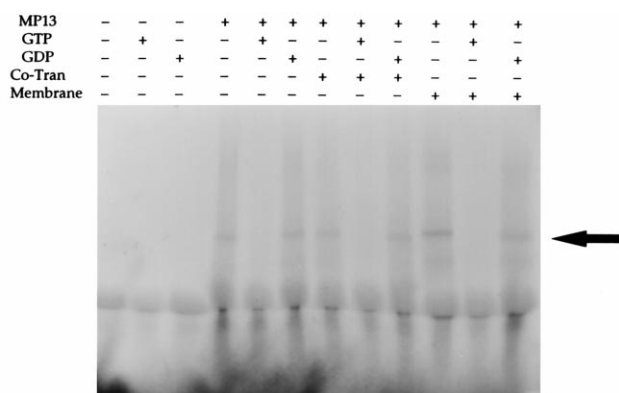


Fig. 4. The *MP13* binding activity to other proteins in the in vitro translation mixture. The *MP13* binding activity to other proteins was identified by gel mobility-shift assay on a 5% non-denaturing polyacrylamide gel. Gels were run at 150 V in 50 mM Tris/50 mM boric acid/1 mM EDTA, dried and autoradiographed. The addition of translated *MP13* products (*MP13*), 100 µM of guanosine 5'-O-(3-thiotriphosphate) for 20 min in the reaction mixture (GTP), and 100 µM of guanosine 5'-diphosphate for 20 min in the reaction mixture (GDP) were showed. Co-Tran represents the addition of reagents before the start of the translation of *MP13*; membrane represents the addition of 1 µl of microsomal membranes in the reaction mixture. '-' Indicates no addition of reagents and '+' is for addition of reagents. The arrow indicates the *MP13* band on the non-denaturing gel. Note that the addition of GTP aborted the *MP13* band in the gel.

the formation of complexes with other molecules acting upstream of SNAREs. Sequence comparisons of *MP13* protein with other members of the rab protein family did not show a high degree of similarity. Since there is no significant similarity within *rabaptin 5*, *rabaptin-3A* [11] and *rab3* [2], it is hard to determine the actual function of *MP13*. However, our gel mobility-shift assay showed that the binding of *MP13* protein to other proteins was GTP related. Further investigation on the interaction of *MP13* protein with *Rab5* and *rabaptin-5* is in progress in order to reveal the function of *MP13*.

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